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A GENERAL DISCUSSION OF TRICHINELLA SPIRALIS (OWEN),
AN INVESTIGATION OF INFECTION OF THE BROWN RAT,
AND A LIFE HISTORY STUDY OF THE PARASITE
IN THE WHITE RAT.

By

Carl A. Johnson

A thesis submitted to the faculty of South Dakota
State College of Agriculture and Mechanic Arts,
in partial fulfillment of the requirements for the
degree of Master of Science, in General Science.

June 1931.

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A GENERAL DISCUSSION OF TRICHINELLA SPIRALIS (OWEN),
AN INVESTIGATION OF INFECTION OF THE BROWN RAT,
AND A LIFE HISTORY STUDY OF THE PARASITE
IN THE WHITE RAT.

Statement of the Problem.

The purpose of this treatise is threefold: first, to present a general discussion of *Trichinella spiralis*; second, to present the results of an investigation of infection with trichinae of 100 brown rats; and third, to present the results of infecting white rats under laboratory conditions.

Under the general discussion of *Trichinella spiralis*, there is included a short history of the discovery of this parasitic worm, a discussion of the normal hosts of the worm, a discussion of its life cycle, a description of the adult worms, larvae, and cysts, an account of the effects of the parasite upon the hosts, treatment of infected hosts, and prevention of infection.

The second section of this treatise presents data on infection with trichinae of 100 brown rats obtained from a number of typical sources. In this section the method used for determining infection in the rats is described. The sources of the rats are discussed, the results of the examinations are given, and finally the conclusion of the investigation is presented.

In the third section of the treatise, the method used in infecting white rats is described. Observations are recorded on

the symptoms observed in the infected rats, and data is presented showing the development of the worms to maturity in the rat hosts, reproduction of the trichinae, migration of the larvae and cyst formation.

The treatise is concluded with a presentation of conclusions and a bibliography of the publications consulted.

History

The trichina worm was first discovered by Peacock in 1828, and in 1835 Owen described it as *Trichina spiralis*. Shortly afterward the worm was found in human beings, both in Europe and North America. Later Leidy discovered it in a hog. Leuckart and others, upon making an investigation, found that the larvae became adults in a few days after they reached the intestines.

The parasite was considered harmless to man until 1860, when Zenker found the adult worms in the intestine of a young girl, who died in a Dresden hospital; it was supposed that she had typhoid fever. The history of the case revealed that she and several others were taken sick immediately after having eaten raw pork from the same hog which contained encysted trichina worms.

From 1880 to 1891, the trichina worm caused international trouble between Germany and the United States. At that time American pork was excluded from the German meat markets because the pork was frequently found to be infected with the parasite. The international complication resulted in the American system of meat inspection.

Occurrence of the Parasite

Trichinella spiralis, commonly known as trichina, is a small parasitic round-worm occurring in its sexually mature stage in the upper part of the small intestine. In the immature stages it occurs as a young larva in the lymph, blood and voluntary muscles, while in its infective stage it occurs as an encysted larva in the voluntary muscles.

The parasite occurs chiefly in human beings, hogs, and rats, tho to some extent in dogs and cats. The worms have been found in many other animals and have been reared experimentally in almost every mammal. They have been found in the intestines of birds, but they do not become encysted in their muscles. Cold blooded vertebrates, as far as is now known, have been found refractory to trichinae.

Life Cycle

Intestinal trichinae are acquired by man because he eats the muscle tissue of pork containing the encysted stage of the parasite in the living condition. The gastric juice of the stomach digests the cyst walls, the encapsulated larvae become freed in the stomach and then pass quickly to the upper part of the small intestine, where they undergo two successive molts. The worms become sexually mature within two or three days after the patient has eaten the pork. In the upper part of the small intestine, the worms mate and the females then penetrate the villi or the mucous membrane of the small intestine, where they

give birth to their offspring. Leuckart has estimated that each female may deposit as many as 1500 larvae during her life.

Since one ounce of sausage meat may contain over 100,000 encysted trichina larvae, most of which develop into females, this small quantity of meat, if eaten, will produce over one million larvae in the body of the victim. The adult trichinae may persist in the intestine for several weeks, but they usually are short lived. The male leaves the host directly after mating, while the female passes out of the host after giving birth to her young.

The different sexes of the adult trichinae vary somewhat in size and shape, the male being $1/12$ to $1/16$ of an inch long and the female $1/6$ to $1/8$ of an inch long (Figures 1 and 2). The worms are whitish in color, cylindrical in shape, slender, and taper from the middle of the body to the anterior end. The two sexes differ in internal structure, but externally they are distinguishable only by the fact that the males are smaller than the females and by the fact that the male has a pair of conical protuberances at the posterior end, which are lacking in the female. The digestive system is very simple. It consists of a muscular pharynx, followed by a long capillary, cuticular oesophagus embedded in a long chain of cells, which occupies the anterior third or half of the body cavity. The intestine which is connected directly to the oesophagus, completes the digestive system. The reproductive system in both sexes occupies a large portion of the body cavity, but there is only one ovary or one testis present. In the male, the opening of the reproductive system and the anus are at the posterior end of the body, while

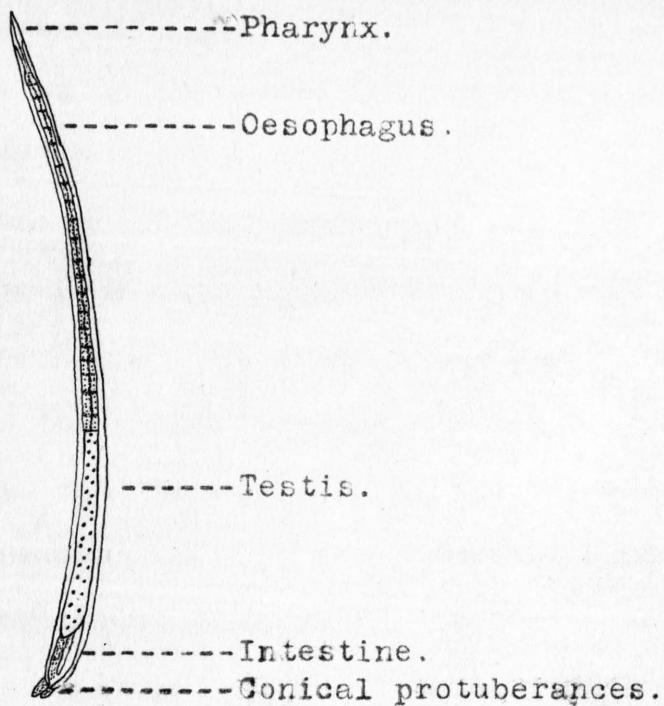


Figure 1.-*Trichinella spiralis*; adult male. (Enlarged).

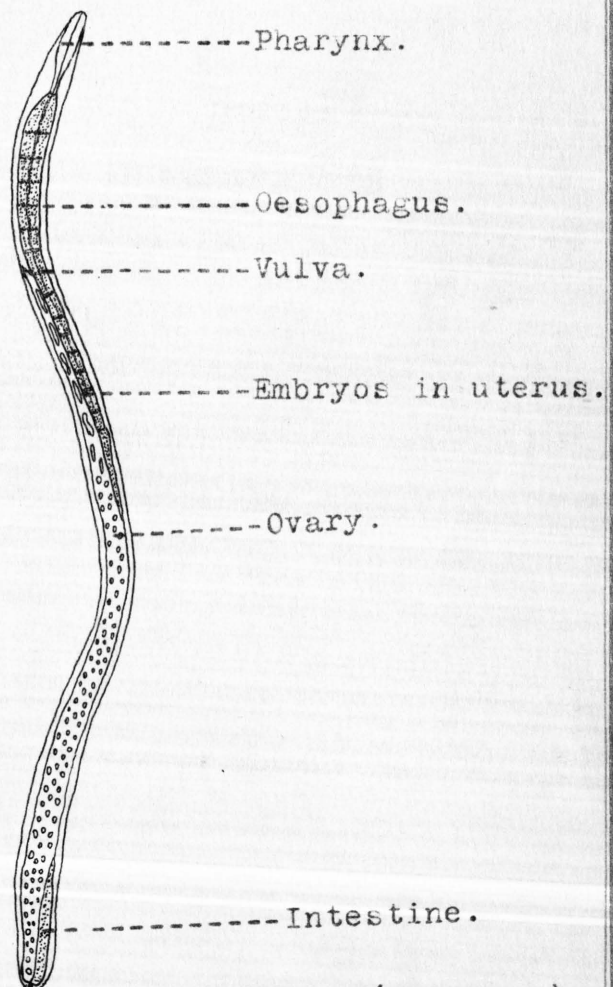


Figure 2.-*Trichinella spiralis*; adult female. (Enlarged).

in the female, the vulva is toward the anterior end and the anus at the posterior end.

The young worms which develop within the female escape from her body in four or five days after mating, or six to seven days after the trichinosed pork was eaten. The young are not nourished within the body of the mother worm as are the young of truly viviparous animals, but the eggs are merely retained in the uterus of the female where they undergo development. Hence the trichinae are really ovaviviparous animals. At birth the young worms are about $1/250$ of an inch long and $1/4000$ of an inch in diameter.

The adult female worm ordinarily buries herself into the villi of the intestine, deep enough so that she lodges her young in the lymph and they are carried by it to the thoracic duct, then to the venous system, and lastly to the heart, from which organ they reach the arterial system. Larvae may also be found free in the small intestine, but in such cases it is doubtful if they ever reach the voluntary muscles. The young worms are most abundant in the circulating blood from the eighth to the twenty-fifth day after infection. The larval worms are carried by the blood to all parts of the body, and in the voluntary muscles they leave the capillaries and penetrate the primary muscle bundles. (Figure 3). The muscles which become most heavily parasitized are the most active ones and those which contain the richest blood supply (as noted by Stäubli), such as the diaphragm, intercostal and laryngeal muscles, tongue and eye muscles. It is thought that the trichina worm subsists on the glycogen stored in the striped muscle, and because the muscles

enumerated have a high glycogen content, they are most readily infected.

When the larvae leave the capillaries in the voluntary muscles, they migrate between the fibers of the muscles and finally each worm penetrates a single muscle fiber. This invasion of the fiber by the immature parasite causes a degeneration, whereby the transverse striae are destroyed. The larva commences to grow within the fiber, which results in the swelling of the adjacent muscle fibers and a thickening of the sarcolemma of the invaded fiber. Here the larva attains the length of $1/25$ of an inch and becomes sexually differentiated at the age of two weeks. The growth of the larva is at the expense of the invaded and surrounding muscle fibers, which are gradually absorbed, while the connective tissue forms the cyst, which surrounds the undeveloped worm. (Figure 4). The cyst may be spherical or lemon-shaped, the former being characteristic of the cysts in rats and the latter in human beings and in hogs. The cysts are from $1/100$ to $1/50$ of an inch long, and when oval in shape, lie parallel to the muscle fibers. Usually each cyst contains only one or two worms, but as many as seven have been found in one cyst. Worms in cysts have been found to live for eleven years in hogs and for twenty-five to thirty-one years in human beings. The beginning of the formation of the cyst capsule occurs within a month after the infection, but it is not completed until seven or nine weeks have elapsed. Six months after the infection, the capsule begins to calcify, but the completion of the calcification process requires at least one additional year.

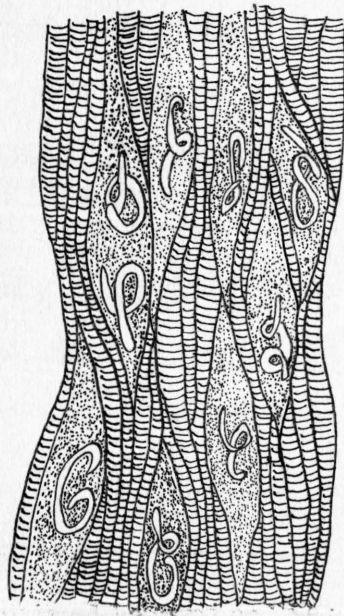


Figure 3.-*Trichinella spiralis*; free larvae in muscle.
(Enlarged).

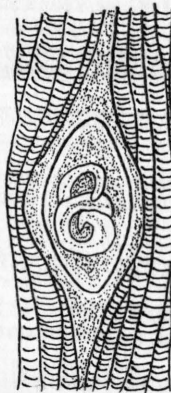


Figure 4.-*Trichinella spiralis*; larva encysted in muscle.
(Enlarged).

Effects of the Parasite upon the Human Being

Human beings acquire the disease, trichinosis, as a result of eating infested, imperfectly cooked pork or products containing muscle tissue of hogs. The severity of the disease depends upon the number of encysted larvae taken into the body. Slightly infested pork, when eaten raw or imperfectly cooked, will produce only a mild form of the disease, providing only a small amount of the pork is eaten. When large quantities of slightly infected pork are eaten a severe attack of the disease may result. If a small or large quantity of heavily infected pork is eaten, an acute attack of the disease will follow.

In severe cases of the disease, three stages may be detected, each corresponding to the behavior of the parasites within the body of the patient. The first of these stages is called the ingression stage, and includes the ingestion of the parasitic worms, the development to maturity of the parasites in the intestine and the production of their brood of larvae. The symptoms of this stage occur from two to three days after infected meat is eaten and last for about a week. The first symptoms of the disease are diarrhea, abdominal pains, intestinal catarrh, and vomiting. These symptoms are caused by the irritation of the intestine, due especially to the activity of the adult female worm. In addition, there may be a feeling of weakness, tension and pain in the muscles. Toward the last of the week puffiness or edema of the face and eyelids appears which may disappear, but occurs later on again.

The second of these stages is called the digression stage and includes the migration and the lodgement of the larvae in the muscles. The symptoms of this stage begin to occur from nine to ten days (not later than two weeks) after the infected meat was eaten, and continue for about four weeks. This stage is characterized by muscular pains and rheumatic aches. The muscles become tense, hard and swollen. Due to the invasion of the larvae in the muscles, it is difficult to chew, to swallow and to move the eye, the eyeball itself becoming inflamed. There is an interference in the respiratory system which may cause asthma or difficulty of breathing. There may be profuse sweating and some fever during this stage.

The third of these stages is called the regression stage and corresponds to the encystment of the parasites. The symptoms of this stage begin to occur about six weeks after ingestion of the trichinosed meat. In the third stage, the symptoms of the second stage become more pronounced, and the legs, forearms, abdominal wall, and face become swollen. The patient becomes anemic, has skin eruption and is likely to acquire pneumonia.

Fever usually sets in early during the first period and reaches its height about ten days after the onset of the disease. Children having the disease are usually exempt from fever. According to Flury, the chemical composition of the infected muscles undergo great changes following the invasion of the fibers by trichina larvae. He considers the substances thus produced to be the cause of fever, muscular pains and edema. As indicated by Herrick, it is quite probable that when the blood and the

tissues become invaded with millions of larvae resulting in the breaking down of large amounts of muscle tissue; a constant inoculation of the infected person with poisonous protein is taking place, which accounts for the fever.

Neurologic symptoms occur when the larvae invade the central nervous system. In this case the larvae may be observed in the spinal fluid, but they do not become encysted and soon perish.

(A) The danger of the disease has passed after the first and (A) heaviest attack of the newly produced larvae, or three to seven weeks after infection. During this time the patient must be able to withstand the irritation and inflammation produced by the burrowing millions of worms and the toxic effect of the chemical decomposition of the parasitized muscle fibers. Recovery usually requires five to six weeks and sometimes several months. Muscular pain and weakness may continue for years, and stiffness may persist indefinitely in the invaded muscles.

Death, if it occurs at all, usually takes place from the fourth to the sixth week after infection, rarely before the second week nor after the seventh week. Trichinosis is more fatal to man than to any other animal. Sometimes thirty percent or more of the severe or reported cases are fatal.

Trichinosis is characterized by a pronounced leucocytosis which is proportional to the severity of the case and a differential count shows a marked eosinophilia, varying from ten to eighty percent. An examination of the blood with these objectives is of great value in diagnosis. Another diagnostic method may be used whereby the embryos may be shown in the circulating blood.

This is accomplished by taking ten cubic centimeters of blood with ten parts of three percent acetic acid, centrifugalizing and then examining the sediment for larval trichinae under the low power of the microscope.

Effects of the Parasite upon the Hog

Hogs acquire the disease by eating infested rats, infested carcasses of other hogs, offal from slaughter houses, and garbage containing scraps of pork. The symptoms are considerably less evident in hogs than in the human beings. In the case of severe infection the symptoms are intestinal disorders, abdominal pains, and stiff muscles.

Treatment and Prevention of Trichinosis

The trichina worms when in the intestine of man are the most difficult to destroy or to remove of all the intestinal worms, because they lodge so deeply in the intestinal tract that ordinary drugs will not affect them. If the disease is detected by a physician within a few hours after the ingestion of the trichinosed pork, he may accomplish some good by attempting to expel the worms from the intestines. The disease is, however, seldom recognized before the larvae are produced. There is no known medical treatment which will affect the embryos of the worms after their migrations have begun.

There is no appreciable immunity conferred after one or more attacks of trichinosis, nor does serum from animals convalescent

from trichinosis when injected into other animals produce an immunity to trichinosis in the latter. (Schwartz, 1917).

Perfect meat inspection and elimination of all infected hog carcasses would result in pork free from trichina worms. Such meat could be eaten raw or partially cooked without any danger of contracting trichinosis, but, as will be noted later, no such practical system of inspection has been attained.

Certain pork products such as the Italian style ham, capicola, and summer sausage, are eaten without cooking and are safe for consumption if prepared in establishments under Federal supervision. These establishments have special methods of processing which consists of cooking and refrigeration. One process consists in heating such products to an inside minimum temperature of 137 degrees Fahrenheit momentarily, a temperature which is sufficient to kill trichinae. Dry sausage or summer sausage must be submitted to one of the following methods: it should either be frozen for 20 days at a temperature not higher than 5 degrees Fahrenheit, or the products should be retained in a dry room for not less than 20 days at a temperature not lower than 45 degrees Fahrenheit.

Salting is also effective if the salt is allowed to penetrate the pork thoroughly and subsequently the meat be dried for three weeks. Due to the fact that the salt withdraws water from the parasites, they are rendered more susceptible to moderately high temperatures and to desiccation. Smoking likewise hastens the killing of the worm.

Concerning pork and pork products obtained from hogs slaughtered on the farm, and those purchased at a local market

that butchers its own hogs, the only safe rule is to cook the pork thoroughly. At such places no scientific methods of refrigeration or pork curing are resorted to, and hence the community is always confronted with the danger of trichinosed pork and pork products.

Meat Inspection

The inspection of the hog carcass consists of a microscopic examination of a piece of muscle from the diaphragm and tongue. If this examination is negative the hog is declared free from the trichina worm.

During the years 1898 to 1906 the United States had compulsory inspection of all the carcasses to be exported. At this time about eight million hogs underwent microscopic inspection, and between one and two percent of the animals were found to be infected. Nevertheless, numerous infected carcasses may have been pronounced trichina free because of the inefficiency of microscopic examination.

No practical system of inspection has yet been devised whereby people who desire to eat raw or imperfectly cooked pork may be safe-guarded from the danger of trichinosis. Experience has shown that microscopic inspection is imperfect and cannot be relied upon as a completely effective preventive measure against trichinosis. Out of 6,329 cases of trichinosis which occurred in Germany between 1881 and 1898, 2,042 (over 32%) were caused by pork which had been inspected microscopically and passed as free from trichinae. While microscopic inspection would in all

probability eliminate most of the heavily infested hog carcasses, many of the lightly infested carcasses would be overlooked. Therefore, raw pork or raw pork products would not be safe for consumption.

Data on Infection of Brown Rats from a Microscopic
Examination of 100 Specimens

Since the brown or wild rat is a host of trichinae, and since hogs may become infested by eating rats, it is important that we know the percentage of rats that are infested with trichinae. Accordingly, a total of 100 rats were procured, some from the Brookings city dump grounds, and some from the vicinity of the local slaughter house. Others were obtained from several farm yards and still others from an isolated granary in the vicinity of Ivanhoe, Minnesota.

Since the muscles of the eye, the diaphragm, the tongue, and the intercostal muscles are most frequently trichinosed, the writer made a microscopic examination of these tissues from each of the 100 rats. In this microscopic examination of the rat tissues, the writer found it convenient to use two pieces of plate glass, each about two inches by six inches. The rat tissues to be examined were placed between these two pieces of glass and then both ends were clamped together. As the clamps were screwed down, the tissues were compressed into very thin sheets. By this means it was possible to get sufficient light to pass thru the tissues so that if trichinae were present, they were readily detected by the use of the low power of compound micro-

scopes magnifying 60 to 70 diameters.

The results of this examination were as follows: of the 36 rats secured from the city dump grounds, 27 or 75% harbored the parasite; of the 17 obtained from the environs of the slaughter house, 14 or 82% were trichinosed; of the 32 from the farm yards, 11 or 34% were infected; and of the 15 from an isolated granary, only 2 or 13% contained trichinae. Thus, a total of 54 or 54% of the 100 rats were trichinosed.

Upon these facts, it may be concluded that the offal and the waste from slaughter houses, which furnish food for the rats of that vicinity, produced the high percentage of trichinosed rats. The rats about the farm buildings get their greatest food supply from the granary, and hence a smaller percentage of rats were found to be infected.

The writer did not attempt to make any comparison as to the severity of the infestation by trichinae of the brown rats. Even if such a comparison had been made, it would not always have shown a relationship to the source, since the rats wander about extensively.

On the average, the writer spent fifteen minutes on the microscopic examination of the tissues of each rat. As such microscopic examination has been unreliable in the past, he wishes to state that altho negative results were obtained with 46% of the rat carcasses which he examined, he would hesitate in declaring them absolutely trichina free, because adjacent tissues which were not examined may have been trichinosed.

Life History of the Parasite in the White Rat

In order to observe the effects of trichinosis on rats, about one cubic centimeter of trichinosed pork was fed to each of five rats. The rats obtained for this experiment weighed from one-half to three-fourths of a pound. During the entire experiment the rats were kept in a wooden cage, with wire netting as a top covering.

During the development of the adult worms, or during the first two to three days after the trichinosed pork had been fed, the rats showed no marked symptoms of the disease. Only slight intestinal disturbances, such as diarrhea, were observed.

The rats were given their usual diet after they had been fed the trichinosed pork. After having been fed this diet for two days, two rats were starved for another two days in order to eliminate intestinal debris. On the fourth day, these two rats were killed and the adult trichina worms were detected in the small intestine. The intestine, after having been removed from the rats, was cut into sections about four inches long. Then the contents were squeezed out of each section by a pair of forceps into a beaker containing some hot 70% alcohol.

Two weeks after the infected pork had been fed, a third rat was killed and the muscles examined. During this period of time, the adult females had given birth to larvae, and most of these larvae had completed their migratory cycle and had lodged in the muscle bundles. They had not, however, penetrated the individual fibers, but were found to be coiling and re-coiling,

slowly winding their way among the muscle fibers.

After three weeks had elapsed since the infested pork was fed to the rats, a fourth rat was killed and the muscles examined under the microscope. It was found that the larvae had penetrated the single fibers and that calcareous cysts had formed around the larvae. After the sixth week, a fifth rat was killed, the muscles were examined, and the cysts had become very pronounced. This is the encysted stage.

Preparation of Permanent Mounts of Adult Trichinae,

Free Larvae and Encysted Larvae.

During the course of the six weeks already discussed, a liberal supply of trichina parasites were obtained and prepared for mounting. The first stage, or the adult worms, were killed by dropping them in hot 70% alcohol. The solution was permitted to cool, but the worms were kept in the solution for 12 hours. After this, they were dehydrated in alcohol of higher concentration, cleared in xylol and finally mounted in balsam on slides. Staining of the worms was somewhat difficult; nevertheless, some were stained in alcoholic eosin and others in Delafield's hematoxylin.

On account of the impermeability of the cuticle of nematodes, Chandler has devised the following method for studying these worms: "The specimens were kept in vials in either 70% alcohol or formalin, the latter preferred. When it is desired to examine them they can be transferred directly to pure 95% phenol, in which they clear perfectly in a few minutes, without any distortion or

shrinkage. Practically all of the anatomic structures, including the details of the lips, mouth capsules, oesophagus, reproductive tubes, intestine, bursa, spicules and papillae can be examined very satisfactorily in this manner. In particularly transparent specimens in which the internal structures are difficult to follow, some advantage can be gained by clearing the worms in a watch glass or syracuse dish containing phenol to which a few drops of carbol-fuchsin has been added. When the worms are lightly stained they should be rinsed quickly in plain phenol and mounted in this; the fuchsin stain slowly diffuses out from the worms, but usually remains long enough to assist in following out the twisting and overlapping tubes."

The second stage consists of the free larvae in the muscles. The muscle tissue was allowed to macerate for about 24 hours in a 2% solution of acetic acid, after which it was teased apart into fibers. The fibers and the larvae between them, were dehydrated in alcohol and mounted in balsam on slides.

In making permanent slides of the encysted larvae the same procedure was used as that given for the free larval stage.

Conclusions

As revealed thru microscopic examination of 100 brown rats by the writer, by scientific facts obtained thru published works and corroborated by the experiments carried on with white rats, it is evident that the rat is the present-day reservoir from which most of our hogs become infested with trichinae. Because of the cannibalistic nature of the rats, the infection is

propagated amongst the rodents. As they wander extensively and are also accidentally transported by ships and freight cars, the distribution of the parasites is almost world-wide. The hog feeds upon the rat and contracts the infection from the rodent reservoir, and finally man is infected by consuming the improperly cooked or cured pork.

To eliminate the danger of trichinosis in man without the proper sterilization, refrigeration or cooking of pork as advocated by the United States Department of Agriculture or Packing Plants operating under Federal inspection, would be a world-wide problem. It would resolve itself into the complete eradication of the host reservoir of the trichina worm, namely, the rat. This apparently is an impossible and an impracticable task.

Under the present conditions, the prevention of trichinosis is a personal responsibility and involves the thorough cooking of pork and pork products. It should be remembered that pork and pork products should be cooked until the minimum interior temperature of 137 degrees Fahrenheit has been reached, the temperature at which trichinae are killed.

Experimentation on the roasting of rib roasts has shown that a rib roast weighing 11.08 pounds requires cooking for 165 minutes or 14.89 minutes per pound at an oven temperature of 257 degrees Fahrenheit, and is then classified as a rare roast. The interior temperature of such a roast when it was removed from the oven was 123.8 degrees Fahrenheit. But a rise of 18 degrees Fahrenheit follows during the next 30 minutes, thus making a maximum interior temperature of 141.8 degrees Fahrenheit

in the roast, or a temperature sufficiently high to kill all trichina larvae. The data here given was taken from Bulletin 242, North Dakota Agricultural Experiment Station, "Standardizing Methods of Roasting Beef in Experimental Cookery," by Esther Latske.

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